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P1: Effect of age, oestradiol levels and endometrial thickness on determining success of frozen embryo transfers

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Human Fertility, August 2008; 11(S1): 1

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Chairman's Welcome

Dear Colleagues and Friends,

The BFS Summer College is scheduled for 2 September 2008–5 September 2008, and will take place at the newly refurbished Adelphi Hotel in Liverpool. This vibrant city is this year's European Capital of Culture and should provide an excellent setting for an exciting academic and social programme.

The College starts with a meeting primarily aimed at Persons Responsible and senior staff in fertility units. I must stress that this day is open to all and, indeed, should be of value to all. Talks by international experts will deal with current contentious issues such as PGD and management of unexplained infertility, with a view to developing a consensus.

The main annual meeting takes as its theme 'The uterus in reproduction'. The central role of implantation in reproduction is clear, and our knowledge of mechanisms involved in successful implantation has developed significantly in recent years. However, for the clinician involved in fertility, failure of fertility treatment at the stage of implantation remains a challenging area. Patients, justifiably, demand answers and a modicum of certainty, but providing these is made difficult by the diverse range of clinical practices and a poorly understood evidence base. We aim to have an exciting programme with well-known speakers covering recent advances in the basic science of implantation and approaches to recurrent implantation failure in the clinic. Special attention is paid to uterine fibroids and their effects on reproduction, including a symposium on the role of uterine surgery in the subfertile woman. We have space for oral and poster presentations of original research from all sections of the membership, and I would encourage particularly younger members and nurses for whom there are a number of prizes. The Summer College will conclude on 5 September 2008 with the Ethics Day, which is always relevant and thought-provoking.

I hope that you will be able to attend the Summer College 2008 and both benefit from and contribute to its wide-ranging and stimulating programme.

Raj Mathur
Chair BFS Meetings Subcommittee

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BFS Summer College 2008

Schedule of Events

Tuesday, 2 September 2008
08:30–09:00 Registration
09:00–17:00 BFS Meeting for Persons Responsible and Senior Staff
18:00–19:30 BFS Summer College Welcome Reception

Wednesday, 3 September 2008
08:30–09:00 Registration
09:00–18:15 BFS Annual Summer Meeting–Day 1
18:15–19:15 BFS Summer College Posters, Cheese and Wine
19:30 Informal Social Evening Dinner, Jalons Restaurant *(Ticket Only)*

Thursday, 4 September 2008
08:00–08:30 Registration
08:30–17:05 BFS Annual Summer Meeting–Day 2
17:05–18:00 BFS Young Clinicians Forum
19:30 BFS Conference Dinner and Awards, Adelphi Hotel *(Ticket Only)*

Friday, 5 September 2008
08:30–09:00 Registration
09:00–17:00 BFS Ethics Meeting

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ABSTRACTS

Fertility 2008
BFS Summer College 2008

OC1: Do patients receiving donor sperm need evaluation of their uterine cavity and fallopian tubes?

Tulay Karasu[1], Ben Lavender[1], Anne Hemingway[2], Geoffrey Trew[1], & Stuart Lavery[1]
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Introduction. In cases of infertility requiring the use of donor sperm, there is a debate about whether investigations for uterine and tubal pathology are routinely necessary, and if so which is the investigation of choice. We wanted to find out whether assessment of the uterine cavity and fallopian tubes with hysterosalpingogram (HSG) detects a significant amount of pathology which could affect treatment outcome.
Material & Methods. This is a retrospective study in a London teaching hospital-assisted conception programme looking into the investigations of women prior to their treatment with Donor sperm. In the time period from January 2003 to November 2007, 162 women underwent assisted conception (IUI/IVF) Donor treatment in our unit. The patients were identified from the embryology database and data were collected from their case notes.
Results. One hundred and forty-nine women (92%) had an HSG performed before starting their treatment. The HSG was normal in 92 women (56.8%) and showed abnormalities in 57 women (35.2%). Uterine pathology only was detected in 31 women (20.8%) and tubal pathology only was described in six patients (4%). Nineteen patients (12.8%) had uterine as well as tubal pathology on HSG examination. HSG was abandoned in one patient due to technical difficulties. Seventeen women (11.9%) underwent laparoscopic surgery, and 10 of these women had confirmed tubal pathology (7% in total), mainly hydrosalpinx. Hysteroscopy was performed in 35 women (24.5%) with 25 women showing uterine abnormalities (17.5% in total). The main findings were polyps in the uterine cavity.
Conclusions. 17.5% of the patients had confirmed uterine abnormalities and 7% of the women demonstrated tubal pathology. The findings of the HSG did influence further management such as the decision to perform surgery or to proceed with IUI or IVF. We therefore believe that evaluation of the uterine cavity and tubes is justified in women before treatment with Donor sperm, and in our own practice we use the HSG.

OC2: Correlation between number of eggs predicted and actual eggs collected during IVF/ICSI stimulated cycles: a prospective observational pilot study

Koli Chandra Reddy, Arianna D'angelo, Grace Jose, Bebbie Jefferies, Lorraine Goucher, & Janet Evans
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Background. It is always difficult to predict accurately the number of eggs to collect during an ART cycle only looking at the follicular size and numbers on transvaginal scan on the day of hCG injection. The accurate prediction is extremely important for patients' expectation and for the laboratory to prepare the culture dishes for the day of the egg collection. The aim is to assess the correlation between number and size of follicles reported by the scan and actual number of oocytes collected.
Material & Method. Prospective observational data collection between December 2007 and February 2008 (6 weeks) at the IVF Wales Unit, University Hospital of Wales, Cardiff.

On the day of the oocyte retrieval, patients' details including demographic factors, stimulation regime, size and number of the follicles on day 11, and when appropriate, day 14 of monitoring were collected. The number of oocytes was predicted on the basis of follicular mean size of $\geq$ 16 mm around the time of the trigger injection. The actual number of eggs was collected on the day of the procedure for each patient. Any difficulties encountered during the procedure (i.e. high ovaries) were noted. Statistical analysis performed using Microsoft Excel software.
Results. Twenty-six (26) women data were collected. Mean age was 34 years (25–42), mean BMI was 26 (19–40), 92.4% were non-smokers, 65.3% were primary subfertile, 65.3% had no previous ART, 23% were PCO, 19.2% had endometriosis, 80.8% used urinary hMG. According to the number of follicles plotted during the scan, 57.6% had good response (6–14 follicles $\geq$ 16 mm), 30.7% had poor response ($<$6 follicles $\geq$ 16 mm) and 11.5% had hyper response ($>$15 follicles $\geq$ 16 mm). On day of hCG injection, 35 follicles were between 12 and 13 mm, 47 follicles were between 14 and 15 mm and 151 were $\geq$ 16 mm (total 233). The total number of eggs predicted was 231, and the actual total number of eggs collected was 217. 69.3% of the procedures were not difficult.
Conclusions. The mean follicular size measured by ultrasound on the day of hCG of $\geq$ 12 mm over-estimates the number of eggs collected by 7.3% (+16 eggs). However, considering only $\geq$ 14 mm on same day underestimates by 8.7% ($-$19 eggs). This can be useful when counselling the patients and for the laboratory organization before the egg collection.

OC3: Effect of pituitary desensitization on the early growing follicular cohort estimated using Anti-Mullerian Hormone

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Background. Although the decrease in FSH secondary to short-term administration of GnRH agonist during IVF does not affect the number of ultrasonographically detected antral follicles, its effect on the early growing follicle population, not evident on even high-resolution ultrasound, is not known. The objective of this study was to evaluate the effect of pituitary desensitization on the early growing

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follicle population through assessment of serum anti-Mullerian Hormone (AMH) concentration. Other markers of ovarian reserve, basal FSH, LH, oestradiol, Inhibin-B and three-dimensional ultrasound ovarian parameters have also been assessed for comparison.
Methods. One-hundred and two subjects aged < 40 years with FSH levels < 12 IU/l underwent venepuncture and transvaginal ultrasound in the early follicular phase of the menstrual cycle and after 14 days of downregulation using GnRH agonists. Serum levels of AMH and other markers of ovarian reserve measured during the early follicular phase and those measured following down-regulation were compared using a paired students' *t*-test for normally distributed or Wilcoxon signed rank test and skewed data, respectively. The study was approved by the National Health Service research ethics committee, and written consent was obtained prior to the enrolment of each subject.
Results. Although mean ($\pm$ SD) AMH levels increased significantly ($P < 0.01$) by about 28% (1.3 ± 0.7–1.6 ± 0.9 ng/ml), there was a significant decline ($P < 0.01$) of about 40–50% in levels of Inhibin-B (47.9 ± 26.5 to 15.0 ± 16.0 pg/ml), FSH (7.1 ± 1.9 to 4.2 ± 1.5 IU/l), LH (5.3 ± 3.0 to 2.6 ± 1.6 IU/l) and oestradiol (156.5 ± 66.3 to 64.3 ± 45.3 pmol/l). Down-regulation treatment was also associated with a decrease ($P < 0.01$) in mean ovarian volume (6.5 ± 2.0 to 5.6 ± 2.2 cm^3) and in ovarian blood flow indices (vascularisation index: 7.5 ± 4.3 to 6.1 ± 5.0; flow index: 36.3 ± 4.7 to 34.1 ± 7.9 and vascular flow index: 2.9 ± 1.8 to 2.3 ± 1.8), but no difference was seen in the antral follicle count (14.9 ± 4.4 to 14.6 ± 6.0).
Conclusion. Pituitary desensitization results in a significant increase in AMH levels, which implies that either the secretion of AMH by early growing follicles is enhanced or that the size of this follicle cohort is increased. The number of antral follicles visualised on ultrasound in the early follicular phase and at down-regulation appears unchanged suggesting any effect is restricted to the smaller 'selectable' follicles.

OC4: Endometrial expression of follistatin and inhibin/activin in women with implantation failure after IVF

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Introduction. The aim of the study was to assess the expression of beta A and beta B subunit of inhibin/activin molecule and follistatin in the endometrium of women with history of implantation failure after IVF and compare it with a fertile control group.
Methods. This was a case–control study. Eleven women with history of implantation failure were recruited from the implantation failure clinic whereas seven women with history of proven fertility were recruited as a control group. All women had daily measurements of luteinising hormone (LH) until an LH surge was identified. An endometrial biopsy sample was then taken at day LH + 7. The tissue obtained was dated using Noyes criteria and immunocytochemistry using the ABC method was performed on paraffin embedded sections to assess expression of beta A subunit, beta B subunit and follistatin molecule expression in the endometrium.
Results. There was a trend for lower beta A stromal score in women with implantation failure although this was not statistically significant. The mean H score for glandular epithelial follistatin expression was significantly lower in women with repeated IVF failure as compared with the control group ($P = 0.03$).
Conclusion. The reduced expression of follistatin in the endometrial glandular compartment in women with implantation failure did not translate into increased activin expression from the endometrium. It may be hypothesized that other factors regulate the activin follistatin pathway than currently known, and follistatin appears to play a key role in implantation.

OC5: Placental dysfunction after infertility treatment

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Introduction. Artificial Reproductive Techniques (ART) and conception following a period of untreated infertility (> 1 year) are independently associated with increased pregnancy complications. Abnormal placentation identified by plasma markers, placental macroscopic and/or microscopic changes may explain some of these variances. The aim of this study was to compare the gestational profile of biochemical markers of placental function and placental histopathology of singleton pregnancies conceived with ART and those conceived spontaneously either with or without a period of infertility (> 1 year).
Methods. Non-smoking, age-matched primiparous women with no significant medical history and with a singleton pregnancy were recruited in three groups: ART ($n = 38$); natural conception ($n = 47$); conception following untreated infertility ($n = 21$). Blood samples were collected at five time points during the pregnancy and tested for soluble fms like tyrosine kinase1 (sFlt1), Placental Growth Factor (PLGF) and Leptin. Placentae were collected and pathological examination was performed by one pathologist blinded to the groups.
Results. ART group had significantly lower plasma levels of PLGF at all time points compared with infertility and control groups ($P < 0.001$). Infertility group had significantly higher levels of leptin than ART or control group at all time points ($P < 0.001$). This did not relate to their BMI. There were no significant differences in sFlt1 levels between groups at the various time points. The mean placental thickness was significantly higher in the ART group ($P = 0.02$) with significantly more placental haematomas ($P = 0.03$) compared with the control and infertility groups. There were no differences in the incidence of abnormal placental shapes or cord insertions. Lesions suggestive of a possible immunodysregulatory response were more prevalent in the infertility group compared with the other groups, but this did not achieve statistical significance.
Conclusion. Low plasma PLGF levels, increased placental thickness and greater incidence of haematomas compared with the other groups suggest abnormal placentation and/or abnormal placental function in ART pregnancies.

OC6: Outcome of cryopreservation of biopsied blastocysts after pre-implantation genetic diagnosis: a 2-year study

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Background. The ability to cryopreserve surplus embryos found to be suitable for transfer after pre-implantation genetic diagnosis (PGD) for serious genetic disease is particularly valuable due to the complex nature and high cost of treatment.
Methods. Between February 2006 and March 2008, we performed 21 PGD thaw cycles, in which 39 blastocysts resulting from embryos biopsied on day 3 were thawed. Outcomes were compared with 142 IVF/ICSI thaw cycles, in which 292 intact blastocysts were thawed, using similar slow freezing and rapid thawing laboratory protocols.

Results. The mean age at cryopreservation was similar in the two groups (32.4 ± 4.7 *vs*. 33.5 ± 3.7 years, $P = 0.21$). There were fewer cryopreserved embryos available for thawing in the PGD cycles (2.7 ± 1.3 *vs*. 4.3 ± 3 frozen embryos, $P = 0.01$). However, the survival rate (87% *vs*. 84%, $P = 0.73$), mean number of embryos replaced (1.5 ± 0.5 *vs*. 1.6 ± 0.48, $P = 0.31$), implantation rate (45% *vs*. 27%, $P = 0.06$) and clinical pregnancy rate (43% *vs*. 34%, $P = 0.38$) per thaw cycle were comparable in the PGD and IVF/ICSI cycles, respectively. In addition, the pregnancy loss rate in the first trimester was similar in the two groups (23.1% *vs*. 23.8%, $P = 0.87$).
Conclusion. Day 3 blastomere biopsy does not compromise survival or implantation potential of cryopreserved blastocysts in PGD cycles.

OC7: An exploration of the views and feelings of women prior to embarking on a cycle of *in vitro* fertilisation who have experienced a previous miscarriage

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Introduction. Miscarriage is an overwhelming experience for any woman, and a cruel blow for women who miscarry after years of infertility treatment. Couples undergoing infertility treatments experience many and varied emotions. This report is an exploration of the views and feelings of women prior to starting a cycle of *in vitro* fertilisation (IVF) who have experienced a previous miscarriage. The findings of this research will enable the care these women receive to be focussed on their needs and the development of the service by better understanding the women's lived experience.
Methods. To address this research problem a qualitative approach is used, and the study is based on phenomenological theory. Ethical approval was obtained. Non-probability, purposive sampling was employed to select the women to be interviewed. The transcribed interviews were analysed using the methods described by Burnard (1991). The categories are: treatment-related factors, psychological factors, pregnancy-related anxiety and suggested ways of improving care.
Results. The women reported concerns about previous assisted conception treatments and shock, anxiety and stress following their miscarriage. They expressed high levels of stress and anxiety about starting treatment again because of the possibility of future loss and grief. Suggested developments in care included: improved patient information, better emotional support, incorporating a telephone help line, support group and counselling and the provision of miscarriage screening 2–3 weeks following their miscarriage.
Conclusion. The data obtained during this project support the findings within the literature that both miscarriage and infertility treatments cause increased levels of stress and anxiety. It confirms previous findings that women experience high levels of stress, guilt, grief and fears about their future childbearing. The women were concerned about starting treatment again and needed support, reassurance and information about treatment and their risk of another miscarriage. They were extremely concerned about the possibility of miscarriage, even before their pregnancy test was positive.

Reference

Burnard (1991). A method of analyzing interview transcripts of qualitative research. *Nurse Education Today*, 11, 461–466.

OC8: The sources of emotional support used by couples/individuals and the place for counselling throughout their IVF treatment

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IVF treatment is regarded as psychologically demanding. Because of this, both the Human Fertilisation and Embryology Authority (HFEA) and the National Institute for Clinical Excellence (NICE) in Britain recommend that all treatment centres offer counselling. However, research has shown that few couples access the counselling provided.
Aims. The aims of this qualitative study were to identify the psychological needs of individuals undergoing such treatment, the perceived effectiveness of any support used and determine both partners' views on the use of counselling.
Methods. Both men and women in 16 couples were interviewed individually following a failed embryo transfer. The results were analysed using a grounded theory approach to categorise the data and develop themes.
Results. It was found that individuals preferred initially to keep their concerns around their infertility treatment private. Men and women were found to use a variety of coping strategies individually but had developed specific strategies for use as a couple. Support from selected family and friends was valued but couples found that coping with family distress was a disincentive to continued family involvement. Individuals found reading about the experiences of others via fertility chat rooms on the internet both helpful and supportive. Printed information and explanations of treatment plans from empathic and friendly staff were appreciated but counselling was only considered to be required when couples felt their situation threatened to overwhelm them.

OC9: *In vitro* maturation: changing practice, setting standards

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The Oxford Fertility Unit made headline news in the national press in September 2007 with reports of the first babies to be born in the United Kingdom as a result of *In Vitro* Maturation (IVM) techniques performed in a UK licensed centre. Although IVM is not a new technique, fewer than 500 babies have been born worldwide, and it has yet to become a mainstream treatment offered by all IVF units in the UK.

The Oxford Fertility Unit was granted a licence by the Human Fertilisation and Embryology Authority (HFEA) to carry out IVM in January 2007, with the first oocyte collection taking place in February 2007. Increasingly, patients are requesting treatment options that involve minimal stimulation regimes or no stimulation at all. To date, 29 cycles of IVM have been completed with an average of 14 oocytes obtained per patient. The average age of women undergoing this procedure is 33 years. 100% of women have had embryos replaced. Positive pregnancy tests have been reported in 34.5% of cycles with an implantation rate of 14% and a clinical pregnancy rate of 24%.

The Oxford Fertility Unit's experience to date demonstrates that with appropriate patient selection, thorough staff training and a great deal of hard work it is possible to offer a successful alternative to standard IVF programmes.

OC10: Ectopic lesions from women with endometriosis show structural and biochemical evidence of delayed maturation

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Following a study on the ultrastructure and lectin histochemistry of eutopic and ectopic endometrium in a model of endometriosis in the baboon (Jones et al., 2006), where initially a delay in differentiation and expression of glycan markers of receptivity was seen, followed in later disease by accelerated maturation, this study aims to describe the ultrastructure and glycan expression in ectopic lesions of women with endometriosis.

Ectopic biopsies from 19 women with proven endometriosis were fixed and processed into epoxy resin for electron microscopy and lectin histochemical examination using *Dolichos biflorus* agglutinin to detect *N*-acetyl galactosamine sequences normally expressed in the mid-to-late secretory eutopic endometrium. Biopsies of eutopic endometrium from 15 healthy women were taken as controls. All patients gave their consent for the study which was approved by the Local Research Ethics Committee (Ref. 06/Q1407/173) and Universities of Manchester and Padua, Italy.

Results showed that, at the ultrastructural level, lesions were very heterogeneous between similarly dated specimens, and showed little resemblance to their normal eutopic equivalents. There was a notable dearth of glycogen accumulation in the second half of the cycle and a complete absence of giant mitochondria and nucleolar channel systems. Many cells lining the lesions resembled mesothelium, and in two cases there was evidence of a migratory cell population which invaded the stroma to form new glandular structures. Almost all lesions failed to express glycans bound by *D. biflorus* agglutinin at the expected mid-to-late secretory phase of the cycle, unlike the controls.

These structural and biochemical findings suggest a failure in differentiation of ectopic lesions which show significant differences in architecture and function from the eutopic endometrium of healthy women. Also, the presence of a migratory cell population in some lesions may have important implications for the aetiopathogenesis of endometriosis.

Reference

Jones, C. L. P., Denton, J., & Fazleabas, A. T. (2006). Morphological and glycosylation changes associated with the endometrium and ectopic lesions in a baboon model of endometriosis. *Human Reproduction, 21*, 3068–3080.

OC11: Diabetes is associated with changes in key regulatory and novel gene expression

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Introduction. It is becoming increasingly clear that far from being innocuous, Diabetes Mellitus is associated with distinct aspects of impaired male reproductive function. With the use of molecular techniques, subtle, yet profound changes in glycation, metabolite levels and sperm nuclear DNA status have all been recently reported by our group. These are changes that have been shown to be associated with decreased embryo quality, lower implantation and increased miscarriage rates. The mechanisms responsible for these perturbations and their consequences remain largely unknown. Because of their transcriptional quiescence, sperm RNA has been considered merely as an artefact. However, the evolution of microarray technology has shown that this previously underestimated resource, acts as an accurate archive chronicling the transcriptional activity involved in the production of the particular sperm sample. We hypothesise that the expression of specific sperm mRNAs is altered in the diabetic state, that these contribute to the damaging changes we have previously found, and that ultimately culminate in the decreased fertility seen in diabetic men.
Aim. By comparing mRNA expression profiles of sperm from diabetic and normal fertile males, to identify which genes were influenced and which mechanisms affected.
Methods. After informed consent was given, semen samples from eight diabetic men were collected, assessed by standard WHO criteria and spermatozoal RNA was then isolated. After confirmation of quality, transcript profiles were obtained by microarray analysis and compared with a database of profiles from fertile men.
Results. The expression of numerous genes was significantly altered in the sperm of diabetic men. Interestingly, many are involved in DNA repair, ROS protection and oxidative stress. Of note was the four-fold increase in the expression of spermatogenesis associated 20 (SPATA 20), a gene that has been localised to the testis but whose function and/or influence remains unknown.
Conclusion. The extent of differences in mRNA expression levels of important regulatory genes in the sperm of diabetic men, provides an indication of the influence and the mechanisms by which Diabetes Mellitus detrimentally affects male reproductive function.

OC12: Effect of coasting on quality and post-thaw performance of cryopreserved embryos

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Introduction. Ovarian hyperstimulation is a potentially life-threatening complication of assisted conception treatment. Coasting is an effective alternative to cycle cancellation in women at high risk of developing OHSS. Various studies have suggested a detrimental effect of prolonged coasting (>3 days) on oocyte and embryo quality and implantation rates. We undertook this study to look at the effect of coasting on embryos after cryopreserved-thawed embryos.
Materials & Methods. The study is a retrospective case control study. Sixty-five patients (39-IVF and 26 ICSI) underwent coasting during the fresh cycle from over 5 years from 2001 to 2005. Sixty-five non-coasted controls were obtained during the same period by matching for type of treatment, basal FSH, main aetiology and body mass index. These 130 women underwent 154 frozen embryo replacement over 6 years between 2001 and 2006. We analysed embryo survival, implantation, clinical pregnancy, live birth and miscarriage rates in the two groups. A further analysis was performed to see the effect of prolonged coasting on cryopreserved embryo performance.
Results. There was no statistically significant difference in post-thaw embryo survival rates (69% *vs*. 66.9%), implantation rates (11.4% *vs*. 16%), clinical pregnancy rates (18% *vs*. 28%), live birth rate (14.4 *vs*. 21.7%) and miscarriage rates (4% *vs*. 6.4%) between the study and control groups. Failed thaw occurred in four cycles in the study and two cycles in the control group. The quality of embryos post-thaw was similar with 59.6% grade I embryos in the coasted cryopreserved group compared with 58.6% in the non-coasted group. On further analysis, there was a trend towards poorer outcome with longer duration of coasting but this did not reach statistical significance.
Conclusion. Coasting does not seem to significantly affect the performance of cryopreserved embryos after thaw. However, there seems to be a trend towards lower implantation rates in the subgroup that underwent prolonged coasting. A much larger, possibly prospective study will be required to further clarify this issue. Our study contains larger numbers than the only published study on this topic in the literature.

OC13: Application of vitrification in the IVF laboratory

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Introduction. Vitrification has the potential to become a very valuable technique for human embryo cryopreservation, and studies have demonstrated high rates of post-warming survival, with healthy children resulting. This service evaluation presents our survival rates and clinical outcomes of cryopreserved human cleavage stage embryos during the first 6 months, following the introduction of vitrification in our centre.
Material & Methods. Eight couples whose embryos had been vitrified following informed consent, attended for frozen embryo transfer. Embryos were vitrified 66–72 h post-insemination in vitrification cooling solutions (Medicult). For thawing, D2 vitrified embryos were warmed in vitrification warming solutions (Medicult) and cultured for 24 h prior to ET. Vitrified D3 embryos were warmed and transferred after at least 2 h of culture.
Results. Twenty-three embryos were vitrified and warmed for these eight couples. Of those, 22 (96.9%) survived post-thaw. Seven patients tested positive for pregnancy (87.5%) via urine analysis after the transfer of two embryos each.

Of these seven pregnant patients, three (42.8%) resulted in a biochemical pregnancy and one resulted in first trimester miscarriage (14.2%). Three clinical pregnancies (2 singletons, 1 twin) were confirmed by the presence of foetal heart and are ongoing at the time of submission. The resulting implantation rate is therefore 25% (4/16).
Conclusions. Vitrification of D2 and D3 cleavage stage embryos on a Cryoleaf is a very promising technique and has been easily incorporated into our embryology laboratory. These early clinical outcomes appear extremely promising and are notably higher than past results with our traditional slow freeze methodologies. Further follow up of the children needs to be undertaken to demonstrate conclusively, the safety and clinical outcome of vitrification.

OC14: Expression and function of fibronectin in male and female gametes during bovine fertilization *in vitro*

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Fibronectin (Fn) is a 440-kDa glycoprotein assumed to play a role in sperm–egg interaction in human. Recently, it has been demonstrated that Fn–when present during bovine *in vitro* fertilization (IVF)–strongly inhibits sperm penetration and fertilization. The present study was conducted (1) to determine which IVF-steps were hindered by supplementation of 500 nM Fn (including its effect on acrosome reaction and sperm motility), and (2) to evaluate the expression of Fn and its integrin receptor ($\alpha5\beta1$) on male and female bovine gametes by means of indirect immunofluorescence. The inhibition experiments indicated that the main inhibitory effect of exogenously supplemented Fn was located at the level of sperm–oolemma binding, with a (concurrent) effect on fusion. In accordance, we detected endogenous Fn underneath the zona pellucida (at the level of the peri-vitelline space) and $\alpha5$ (subunit of Fn-receptor integrin $\alpha5\beta1$) on the oolemma of cumulus-denuded bovine oocytes. In addition, all sampled bovine sperm cells displayed integrin $\alpha5$. In the non-treated and capacitated sperm fraction fluorescence at the rostral sperm head was observed, whereas a fluorescent band at the equatorial segment was noted in the acrosome reacted spermatozoa. The Fn molecules observed at the surface of non-treated and capacitated spermatozoa seemed to disappear during acrosomal reaction. Moreover, incubation of sperm cells with Alexa Fluor® 488-conjugated Fn also resulted in green fluorescence at the equatorial segment. Combining these results, the inhibitory effect of exogenously supplemented Fn seemed to be exerted on the male gamete by binding to the exposed integrin $\alpha5\beta1$ receptor after acrosome reaction.

The presence of endogenous Fn underneath the zona pellucida together with integrin $\alpha5$ expression on the oolemma and the acrosome reacted sperm cell surface, suggests interaction between the Fn ligand and corresponding receptors on both (acrosome reacted) sperm cell and oolemma, initiating sperm–egg binding. Further research, identifying the effect of Fn binding to its integrin $\alpha5\beta1$ receptor on the intracellular signal transduction in male and female gamete, is indispensable to elucidate the exact underlying mechanism of interaction in order to validate our model and to create a non-hormonal topical contraceptive – based on the glycoprotein – in the future.

OC15: Direct health service costs of providing assisted reproduction services in older women – retrospective cross-sectional analysis

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Objective. To assess the total service costs incurred for each live-birth achieved by older women undergoing IVF (*in vitro* fertilization) in comparison with costs in younger women.
Design. Retrospective cross-sectional analysis.
Setting. IVF unit and maternity hospital in a tertiary care setting.
Participants. Women (1854) who underwent their first cycle of IVF between 1997 and 2006. Of these, 341 (18.4 %) were under 30, 714 (38.5%) were 30–34; 640 (34.5 %) were 35–39 and 159 (8.6%) were 40 years or more.
Intervention. Bottom up costs was calculated for all interventions in the IVF cycle. Early pregnancy and antenatal care costs were obtained from NHS reference costs, ISD Scotland and local departmental costs.
Main outcome measure. Cost per live birth in each age group. Secondary outcome measures included cost per pregnancy and cost per ongoing pregnancy.
Results. The mean cost per live birth (95% CI) in women undergoing IVF at the age of 40 years and above was £37,824 (£26,911–£64,962), which is more than 2.5 times higher than those aged 35–39 years (£17096 CI, £15,635-£18,937). The cost per ongoing pregnancy was almost three times in women aged 40 and above (£31,642; 95% CI, £21,241–£58,979) when compared with women 35–39 years of age (£11,300 CI, £10,006–£12,938).
Conclusion. The cost of a live-birth following IVF rises significantly at the age of 40 due to lower success rates. Most of the extra cost is due to the IVF treatment, but some of it is due to higher rates of early pregnancy loss.

OC16: Preimplantation genetic diagnosis for the prevention of sickle cell disease: current trends and barriers to the uptake of this service at Guy's & St. Thomas NHS Foundation Trust

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Introduction. Sickle cell disease (SCD) is a potentially debilitating haemoglobinopathy with increasing global incidence. In the absence of effective curative therapies, preventative measures such as preimplantation genetic diagnosis (PGD) are being employed to reduce the incidence of the disorder. We describe the experience

of the use of PGD for the prevention of SCD at a tertiary referral PGD centre in a London teaching hospital and explore the potential barriers to the wider uptake of this service.
Methods. A review of 16 PGD cycles performed for the prevention of SCD in 12 couples at risk of having an affected child was conducted. We also compared the outcome of PGD for SCD and PGD for other autosomal recessive disorders (ARDs) involving 122 cycles performed during the same period.
Results & Discussion. Two clinical pregnancies resulting in the live birth of two unaffected children were reported for the 16 PGD cycles carried out (clinical pregnancy rate of 13% per initiated cycle). The data for PGD for the other ARDs showed a 25% clinical pregnancy rate per initiated cycle. In addition, the fertilisation rate was significantly higher in PGD for the other ARDs (58%) compared with PGD for SCD (42%).

The uptake of this service at GSTT seems relatively low compared with that of PGD for other monogenic disorders such as cystic fibrosis. Possible explanations for this observation include a general lack of awareness of the service among patients and local healthcare providers and changing public attitudes to SCD due to enhanced therapeutic management and coping strategies. In addition, the high cost of the procedure coupled with a suboptimal success rate may deter some at-risk couples from undergoing the procedure.

PGD for the prevention the birth of a child affected by SCD is an established, viable treatment option for couples at risk of having an affected child. However, barriers to uptake of this service need to be fully addressed in order to ensure its availability to all couples seeking to avoid having child affected with SCD.

OC17: Non-blastocyst selective single embryo transfer does not reduce success rates in ART cycles in women at high risk of multiple pregnancy

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Objective. To determine the clinical pregnancy rate (CPR) and multiple pregnancy rate (MPR) in an ART programme following the introduction of a new selective single embryo transfer protocol in a selected group of women undergoing fresh IVF/ICSI treatment cycles.
Population. 420 fresh ART cycles were performed between October 2007 to April 2008 at the Assisted Conception Unit. Of these, 41 women were eligible for the new protocol ($\leq$35 years old with excess embryos for freezing Group A) and 73 were excluded ($\leq$35 years old with no excess embryos for freezing Group B).
Methods. A new protocol was introduced following our HFEA inspection 2007 and in line with the HFEA recommendation of an MPR $<$ 10% by 2010. This included a multidisciplinary approach with patients being educated about the risks of multiple pregnancy following the transfer of more than one embryo. The aim was to transfer day 2 or 3 post-egg collection embryos without the need for a change in our working pattern.

Main outcome measures CPR and MPR in eligible and non-eligible women undergoing a fresh ART cycle.
Results. This selective single embryo transfer protocol resulted in no difference in CPR 39% (16/41) in Group A *versus* 37% (27/73) in Group B and a reduction in MPR from 30% (8/27) in Group B to 0% (0/16) ($P = 0.045$) in Group A, with an overall MPR of 19% (8/43) in Groups A + B for this age group.
Conclusion. Selective single embryo transfer in women with prognostic features of $\leq$35 years and embryos available to freeze should be mandatory. This protocol change can be introduced as routine practice for all day 2 or 3 embryo transfers without the need to change the clinic working pattern. As this is the age group associated with the highest MPR this represents a significant step towards achieving the HFEA goal of $<$10% MPR. We are in the process of reviewing our protocols to reduce this further.

OC18: Egg donation before and after April 2005

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Introduction. In April 2005, UK legislation was changed requiring any donor of gametes or embryos used in the treatment of other people to agree to the disclosure of their identity to any offspring on reaching the age of 18.
Method. A retrospective study to find the effect of the change in law on the egg donation programme in the UK's largest fertility unit at Liverpool Women's Hospital.
Results. Forty-eight women donated eggs between April 2005 and April 2008, out of which 40 were known donations and 8 were anonymous donations. Eighteen women approached their sisters to donate eggs and another 24 took help of their 'friends'. Five women donating eggs anonymously, donated to two recipients.

Compared with 3 years before April 2005, 45 women donated eggs, of which 15 were known donation and 30 were anonymous. In the known donor group, 12 were sisters of women needing eggs, one was sister-in-law and two were friends. Twenty women donating eggs anonymously donated eggs to two recipients.

Currently, there are 86 women awaiting egg donation in our unit. Considering the long waiting list and shortage of egg donors, we have introduced egg-sharing programme in our unit since April 2008. Out of these 86 women, 40 are considering egg sharing.
Conclusion. Our results show the effect of change in law had on an already restricted egg donation in UK. There is a need to address this egg shortage by developing gamete donor recruitment strategies.

P1: Effect of age, oestradiol levels and endometrial thickness on determining success of frozen embryo transfers

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Introduction. Frozen-thawed embryo transfer (FET) is a widely used technique, with replacement of embryos during natural menstrual cycles or in cycles constructed by exogenous steroids having similar outcomes in many series. Multiple factors have been proposed to have a positive influence on the success rate of FET, including increased endometrial thickness, younger maternal age and lower circulating oestradiol. The impact of these variables in the context of constructed cycles, using GnRH agonists to facilitate suppression of ovarian function prior to exogenous steroid administration, is not known. The aim of this study was to determine the predictive ability of these indices on clinical outcomes.
Material & Methods. Charts of consecutive patients undergoing constructed cycles of FET between February 2005 and September 2007 were reviewed. Construction of FET cycles was by mid-luteal administration of depot GnRH agonist, with confirmation of down-regulation 2–3 days after menstruation (serum oestradiol $<$ 200 pmol/l and endometrial thickness $<$ 5 mm), prior to initiation of oestradiol valerate 6 mg/day. Transvaginal ultrasound and biochemical monitoring of oestradiol were performed on days D8 and D13, and progesterone 800 mg/day commenced on D13 if endometrial thickness $\geq$6 mm. Patients failing to meet this criterion were reassessed on day 18. Oestradiol was measured by Immulite with intra- and inter-assay CVs of 8.6% and 9.3%, respectively. Results are presented as mean $\pm$ standard deviation or unadjusted median (interquartile range). Pearson correlations, general linear models and stepwise logistic regression ($P \leq 0.15$ for inclusion of predictors) on log transformed variables were used to assess relationships.

Results. One hundred and fifty seven women with a median age of 35 years (31–37 years), median oestradiol of 215 pmol/l (120–305 pmol/l) and median endometrial thickness of 8.5 mm (7.2–9.9 mm) underwent a constructed FET. Biochemical pregnancy test was positive in 60 (38.2%), with 34 (21.6%) achieving a clinical pregnancy as defined by positive fetal heart beat at 8 weeks gestation. There were no significant differences in age, peak oestradiol levels or endometrial thickness in those women achieving a biochemical or clinical pregnancy. Despite this, women with endometrial thickness ≥ 8 mm were significantly more likely to achieve a clinical pregnancy (<8 mm 13.1%; ≥ 8 mm 27.1%; $P=0.04$), although no differences were observed for positive pregnancy tests (<8 mm 32.8%; ≥ 8mm 41.7%; $P=0.26$). Age and endometrial thickness were positively associated ($r=0.18$, $P=0.02$), with circulating oestradiol not related to endometrial thickness ($r=0.10$, $P=0.19$) or age ($r=0.11$, $P=0.18$). Peak oestradiol (≥ 225 pmol/l) predicted biochemical pregnancy better than age ($P=0.024$) or endometrial thickness ($P=0.049$), however, it still performed relatively poorly with an area under the ROC curve of 0.66 (95%CI 0.58–0.74), sensitivity 65% (95% CI 51.6–76.9%) and specificity 64.9% (CI 54.6–74.4%). Age (AUC 0.56 (95%CI 0.48–0.64)), peak oestradiol ((AUC 0.55 (95%CI 0.47–0.63)) and endometrial thickness (AUC 0.58 (95%CI 0.50–0.66)) all performed equally poorly in the prediction of clinical pregnancy. Multivariate analysis demonstrated that only peak oestradiol was independently associated with a positive pregnancy test (contribution to variance 6.4%, positive, $P=0.001$), however, this relationship was not sustained for prediction of clinical pregnancy.
Conclusions. We demonstrate that endometrial preparation with oestrogen and progesterone after pituitary desensitisation with a depot GnRH agonist is an effective protocol in women undergoing FET, and achieves pregnancy rates similar to those reported following fresh replacement cycles. Age does not affect the pregnancy rate in women undergoing FET; similarly peak oestradiol does not predict clinical pregnancy rates and is not a useful additional test in women undergoing FET. The lack of relationship between endometrial thickness and pregnancy outcome questions the assignment of a lower limit prior to embryo replacement.

P2: A randomized controlled trial of tubal flushing for unexplained infertility

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Background. There has been debate in the literature for over 40 years as to whether hydrotubation of the fallopian tubes enhances fertility and whether this presumed therapeutic role is greater with oil-soluble contrast media than with water-soluble contrast media used for hysterosalpingography (HSG).
Aims. To evaluate the influence of the contrast material used in HSG on subsequent reproductive success.
Method. A prospective, randomized, single blinded controlled trial. Seventy-two couples with a diagnosis of primary or secondary unexplained infertility were recruited in our trial. Computer-generated random table were used and patients randomized before HSG procedure. HSG was performed primarily as a diagnostic procedure and was done within 10 days following menses. Thirty-five couples were randomized to the water soluble group and thirty-seven to oil soluble group. Four women were withdrawn after randomization, as in two HSG could not be performed due to technical problems and two women had bilateral blocked tubes. Couples were followed up for 4 months and diagnosis of pregnancy was confirmed by a trans vaginal ultrasound.
Results. Eight (21%) got pregnant in oil-soluble group and in three of these women initial resistance was experienced during tubal flushing. In water-soluble group five (14%) got pregnant but two had early miscarriage.
Conclusion. Our study favours the effectiveness of tubal flushing with oil soluble contrast media in increasing odds of pregnancy. This could represent a simple, less invasive and economic alternative to IVF for women with normal fallopian tubes.

P3: Salpingitis following oocyte aspiration and embryo transfer – a case series

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Introduction. Salpingitis following oocyte aspiration and embryo transfer is a recognized rare complication of *in vitro* fertilization therapy. The reported incidence is between 0.3 and 0.5%. We report two cases of pyosalpinges in a series of more than 2000 transvaginal egg collection and embryo transfer procedures.
Case reports. Two women undergoing *in vitro* fertilization treatment for tubal factor infertility presented with pyosalpinges at Countess of Chester Hospital.

Case 1, JW, developed severe abdominal pain 2 days after frozen embryo transfer during natural cycle *in vitro* fertilization. The pyosalpinx was initially drained percutaneously under ultrasound guidance and resolved on intravenous antibiotic therapy. Laparoscopic salpingectomy was carried out 10 weeks later to remove a persisting hydrosalpinx.

Case 2, BL, had ultrasound guided transvaginal egg collection under antibiotic cover. She presented 15 days post-embryo transfer with abdominal distension and peritonitis. Laparatomy revealed bilateral suppurative pyosalpinges and several loop abscesses.

In both cases, high vaginal and endocervical swabs were taken prior to initiation of treatment and were negative.
Discussion. The possible mechanisms for development of pyosalpinx following egg collection and embryo transfer include:

(a) Puncture of pelvic viscera at the time of egg collection.
(b) Rekindling of quiescent infection within the tubes from a previous infection.
(c) Introduction of vaginal flora into the pelvic cavity during oocyte aspiration.
(d) Introduction of organisms during embryo transfer.

In our unit, one dose of intravenous cefuroxime is given prophylactically during egg collection but not at embryo transfer.
Conclusion. These cases highlight the risk of serious complications associated with *in vitro* fertilization therapy, emphasising the need for appropriate counselling pre-treatment.

The possibility of severe pelvic infection following *in vitro* fertilization therapy warrants consideration of prophylactic antibiotic cover.

P4: Costs and outcomes associated with mild *in vitro* fertilisation or intracytoplasmic sperm injection using recombinant follicle stimulating hormone

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Background & Objectives. In 2004, based on clinical evidence and economic analysis, the National Institute for Health and

Clinical Excellence (NICE) recommended that the NHS should fund up to 3 cycles of *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) for most women requiring treatment. This recommendation was not fully implemented by government, citing budget concerns, despite NICE finding IVF/ICSI to be effective and cost-effective use of resources. This study assesses cost and outcomes of IVF/ICSI in the UK, in usual practice in the UK.

Methods. The study data utilised for this analysis is taken from anonymised audit data held by a UK assisted conception unit (ACU) unit. The data held in the ACU dataset covers women receiving fresh cycles of IVF/ICSI from October 2001 to January 2006 in the unit which treats both National Health Service and privately funded patients. Women undergoing IVF or ICSI who were treated with one brand of recombinant follicle stimulating hormone (rFSH) (Puregon®) within the ovarian stimulation protocol were included in this analysis. Rates of ongoing pregnancies per cycle and live birth rates were calculated. Drug use was determined by linking ACU data to pharmacy dispensing data. Costing for clinic procedures was based on a prior financial audit of the centre. Costs were applied at UK 2007 prices.

Results. Data were available and analysed for 1418 IVF/ICSI cycles undertaken by 1001 women. Mean duration of ovarian stimulation was 9.1 days (95%CI: 9.0–9.3 days). The clinical pregnancy rate/cycle was 36.4% (95%CI: 33.9–39%), the ongoing pregnancy rate was 24.4% (95%CI: 22.2%–26.7%), and the live birth rate was 22% (95%CI: 19.7–24.2%). The average rFSH dose/cycle prescribed was 1855 units (SD: 561), whereas the average dispensed dose/cycle was 1891 units (SD: 540). The average cost of rFSH/cycle was £646 (SD: 219). Average cost/cycle for concomitant medications and procedures was £159 (SD: 122) and £2,127 (SD 349), respectively. The average total cost/cycle was £2,932 (SD: 422). The average cost per clinical pregnancy, cost per ongoing pregnancy and cost per live birth were £8,058, £12,017 and £13,326, respectively.

Conclusions. Although IVF/ICSI outcomes in this usual practice setting were similar to UK averages the cost of rFSH/cycle was lower than that estimated in the 2004 NICE guidelines. These findings suggest that budgets for IVF/ICSI can be optimised taking efficient drug delivery practices into account. However, the UK government should increase funding overall to cover the recommended levels of IVF/ICSI.

The study was funded by Organon, part of Schering-Plough Corporation.

P5: Process mapping: investigation into annual number of new patients required to successfully generate a patient list for IVF/ICSI

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Introduction. Not all patients referred to an infertility clinic continue on to receive treatment, either due to eligibility or personal circumstances. This study aims to follow NHS patient numbers from a new patient clinic, to the waiting list, to those that attend an evening information meeting and eventually receive treatment. This will demonstrate where most dropouts occur and the number of new patients required to generate a treatment list.

Material & Methods. Data from new patient clinics from April 2006 were obtained along with the number of patients who are on the waiting list. As the duration of time on the waiting list is 1 year (Welsh Assembly Government Criteria), numbers of patients who attended evening clinics and received treatment the following year were obtained until March 2007 to ensure follow up of the same patients.

Results. As expected there were multiple dropouts at each stage. 707 patients attended new patient clinics between April 2006 and March 2007, and of these 268 received treatment for IVF/ICSI, giving a 37.9% uptake. Further analysis of the results will be required to determine where these dropouts are occurring most frequently.

Conclusions. There is an obvious discrepancy between the numbers of patients presenting to new clinics who continue on to receive IVF/ICSI treatment. This study shows in order to obtain a patient list of 100 patients for IVF/ICSI treatment initially 264 patients will need to be seen. A prospective study will follow this pilot to explore reasons for the findings.

P6: Is a reliable ovarian response predictable with AMH and antral follicle count–can we reduce cycle cancellation in poor responders irrespective of age? A retrospective analysis

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Background. Controlled ovarian stimulation is still considered as one of the advances in the field of infertility medicine. The dose of stimulation medication is based on a number of factors grouped together and called as the ovarian reserve tests or ovarian markers for stimulation.

The most widely used endocrine marker for ovarian reserve is the early follicular phase FSH level. Early follicular (basal) FSH level has been shown to be an independent predictor of IVF outcome. Even though there are other markers of ovarian response, the reliability on any single determinant was of doubtful significance.

The availability and application of so many tests of ovarian reserve serves to illustrate the lack of a single reliable technique. However, a recent addition to the list of promising candidates for predicting ovarian response is anti-Mullerian hormone (AMH), a member of the transforming growth factor-β super family. AMH is produced in the ovary by granulosa cells of growing preantral and small antral follicles.

Materials & Methods. This retrospective review included a total of 138 patients classified as poor responders. All patients had AMH measured on day 4–6 of their menstrual cycle along with a transvaginal scan to assess the Antral follicle count. Patients who underwent IVF/ICSI cycle between February 2006 and March 2008 were included in this study. The stimulation regimen and dose was based on two ovarian markers.

Results. One hundred and twenty eight patients of this study group underwent oocyte retrieval, and 120 of them underwent embryo transfer.

Conclusion. AMH and antral follicle can be considered as best two indicators for poor responders irrespective of age. No significant association with the ovarian markers and the outcome of IVF/ICSI treatment were evident.

P7: The introduction of anti Mullerian hormone in an IVF setting

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Background. Anti-Mullerian hormone (AMH) has shown to be better predictor of ovarian reserve than follicle stimulating hormone (FSH), antral follicle count, inhibinB and age. Diminishing ovarian reserve is a common occurrence in the IVF setting. Prediction of response to IVF and optimizing the ovarian stimulation regime to avoid cancellation is of great importance. Also AMH may be useful in avoidance of cancellation due to excess response (ovarian stimulation syndrome OHSS).

Objective. To look at our initial experience of the introduction of AMH levels prior to an IVF cycle and its correlation with outcome, thus leading to the development of protocols for the determination of appropriate dosage of gonadotrophin for individuals.
Design. Ongoing audit of the introduction of AMH and correlation of levels with outcome since January 2008. Review of case notes to correlate the ovarian response, clinical pregnancy rates and OHSS.
Results. So far 60 patients have had AMH levels assessed. Amongst the patients with AMH less than five, (33%) had a poor response (less than five follicles). 10% had failed fertilization and 45% had a negative pregnancy test. Of those, seven patients with high AMH levels (>15), five (71%) had confirmed OHSS and all needed cancellation of cycles or 'freeze all' embryos.
Conclusion. The introduction of AMH measurement in our unit looks promising. Compared with FSH, AMH can be done at any time during the menstrual cycle, and therefore is convenient. In addition, measurement of AMH has been demonstrated to be useful in detecting those women at risk of a suboptimal outcome, in whom gonadotrophin dosage may now be optimized. Protocol development has been undertaken and will be implemented and audited.

P8: Application of vitrification in the IVF laboratory

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Introduction. Vitrification has the potential to become a very valuable technique for human embryo cryopreservation, and studies have demonstrated high rates of post-warming survival, with healthy children resulting. This service evaluation presents our survival rates and clinical outcomes of cryopreserved human cleavage stage embryos during the first 6 months, following the introduction of vitrification in our centre.
Material & Methods. Eight couples whose embryos had been vitrified following informed consent, attended for frozen embryo transfer. Embryos were vitrified 66–72 h post-insemination in vitrification cooling solutions (Medicult). For thawing, D2 vitrified embryos were warmed in vitrification warming solutions (Medicult) and cultured for 24 h prior to ET. Vitrified D3 embryos were warmed and transferred after at least 2 h of culture.
Results. Twenty-three embryos were vitrified and warmed for these eight couples. Of those, 22 (96.9%) survived post-thaw. Seven patients tested positive for pregnancy (87.5%) via urine analysis after the transfer of two embryos each.

Of these seven pregnant patients, three (42.8%) resulted in a biochemical pregnancy and one resulted in first trimester miscarriage (14.2%). Three clinical pregnancies (2 singletons, 1 twin) were confirmed by the presence of foetal heart and are ongoing at the time of submission. The resulting implantation rate is therefore 25% (4/16).

Conclusions. Vitrification of D2 and D3 cleavage stage embryos on a Cryoleaf is a very promising technique and has been easily incorporated into our embryology laboratory. These early clinical outcomes appear extremely promising and are notably higher than past results with our traditional slow freeze methodologies. Further follow up of the children needs to be undertaken to demonstrate conclusively, the safety and clinical outcome of vitrification.

P9: High ongoing pregnancy rates with a new vitrification medium after oocyte vitrification and thawing

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Vitrification of oocytes is a relatively new method and its superiority over slow freezing is under debate. More vitrification media became commercially available compared with several years ago. The present study reports outcome of cycles in which oocytes were frozen and subsequently thawed (and transferred) with a recently introduced vitrification media (SAGE Biofarma, Thornbull). Metaphase II oocytes were frozen in SAGE Vitrification Media at $\sim$3 h after retrieval immediately after denudation. The vitrification procedure was performed according to the Kuwayama protocol (Kuwayama et al., 2005) using the Cryotop technique in high security straws (CBS, Paris). The thawing procedure has been performed as described previously (Kuwayama et al., 2005). In seven donor cycles, transfer embryos were obtained from oocytes that have been vitrified and subsequently thawed (Table 1). From 96 vitrified Metaphase II oocytes, 69 survived (% mean $\pm$ SD; 69 ± 18) and were subsequently subjected to ICSI. The fertilization rate was 71 ± 26 (%). All cycles resulted in embryo transfer. The results have been obtained in four transfers of whom three were positive and all are currently ongoing (between 9 and 15 weeks). The implantation rate was 36% (4/11). These results showed that high ongoing pregnancy rates can be obtained after vitrification and thawing of oocytes in SAGE media. The survival rate can be improved after an extended time period when the embryologists get experienced and by reducing the number of oocytes that were subjected to vitrification in each straw.

Reference

Kuwayama, M., Vajta, G., Kato, O., & Leibo, S. P. (2005). Highly efficient vitrification method for cryopreservation of human oocytes. *Reproductive Biomedicine Online*, 11, 300–308.

Table 1. Outcome of cycles in which embryos derived from vitrified and thawed oocytes were transferred.

ET cycle	Frozen oocytes	Survived oocytes	% Survival	Two pronuclei oocytes	% Fertilization	Number of embryos transferred	Day of embryo transfer	Number of sacs
1	8	7	88	3	43	2	2	1
2	12	6	50	5	83	4	2	1
3	9	7	78	2	29	2	2	0
4	7	3	43	3	100	3	3	2
5	20	18	90	16	89	4	3	NA
6	24	15	63	11	73	3	3	NA
7	16	12	75	10	83	2	6	NA

NA, The result of the β-hCG test is not available yet.

P10: Effect of implementation of NICE guidelines on BMI with regards to IVF/ICSI success rates

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Introduction. NICE introduced a recommendation that women's BMI should be between 19 and 30 prior to commencing IVF for optimal success rates and this was implemented in Wales by the Welsh Assembly Government in June 2006 for all NHS patients. This study aims to evaluate whether this change was associated with any change in clinical pregnancy rates.
Material & Methods. A database was available with in the Cardiff Assisted Reproduction Unit that enabled a comparison of clinical pregnancies rates and BMI between data from 2005 and 2007 (excluding 2006 when the implementation began to be introduced). Data regarding pregnancy rates and BMI was studied from 2005 to 2007.
Results.

	% Clinical pregnancy rates	% Women BMI > 30
2005	14.9	16.9
2007	31.1	2.3

Conclusions. Implementation of the NICE guidelines was associated with a dramatic improvement in pregnancy rates.

In a retrospective study such as this, other changes may be happening simultaneously. In particular, non-smoking criteria, and a reduction in the waiting time, making patients slightly younger at the time of treatment, were also introduced by the Welsh Assembly Government, and these factors, together with laboratory changes, may have played a part in the improvement. A more complete analysis of the data is planned looking at these additional demographic factors.

P11: Spontaneous conception rates are increased during monitored cycles in couples with a conception delay

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Measurement of the mid-luteal phase serum progesterone is a standard fertility test to confirm that ovulation has occurred. However, formal cycle monitoring with scans during the early follicular phase, pre-ovulation and mid-luteal is educational for patients and can help reveal subtle signs of reduced ovarian reserve, such as dysfunctional follicular development. For some couples, there is the additional benefit of knowing that ovulation is imminent. Cycle monitoring is offered to all women with delayed conception as a routine preliminary investigation in our unit. We retrospectively audited the outcome of 61 monitored cycles and compared pregnancy rates in the monitored cycle with that of the subsequent five unmonitored cycles. (This study constituted an audit of standard clinical practice and therefore did not require ethical approval).
Methods. Consecutive natural cycles monitored between 1 January and 31 December 2006 were included in the study where the outcome of the cycle was known, i.e. date of subsequent period or result of a pregnancy test. The number of pregnancies occurring over the next 5 months was recorded.
Results. Three hundred and seventy three cycle monitoring records were assessed of which complete data was available on 61 women. Of these, 49 women ovulated and 12 did not. Seven women conceived during the monitored cycle (14.3%) and four women conceived over the next five cycles (10.2%, unmonitored cycles). There was no difference in the mean age of the women who conceived compared with those who did not (conceived: mean age 35 years, range 29–41 years; not pregnant 35 years, range 23–46 years).
Discussion. Formal cycle monitoring is a useful fertility investigation and may have an additional advantage of increasing spontaneous conception rates, probably by encouraging intercourse in the days leading up to ovulation.

P12: The optimal dose of human chorionic gonadotrophin for final oocyte maturation and ovulation in *in vitro* fertilization

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Background. Although the human chorionic gonadotrophin (hCG) has been established as the routine oocyte maturation trigger in *in vitro* fertilization (IVF), there is no consensus over the acceptable dose that should be used for final follicular maturation.
Objective. To identify the optimal dose of urinary hCG (u-hCG) that can trigger final oocyte maturation and ovulation without increasing the risk of ovarian hyperstimulation syndrome (OHSS).
Design. We conducted a systematic review considering all controlled studies, both prospective and retrospective that assessed the effect of at least two different doses of u-hCG administered for final oocyte maturation, on IVF outcome and on the incidence of hCG-related adverse effects. Both agonist and antagonist cycles were considered.

The primary outcome was the live birth rate. The secondary endpoints included the pregnancy and clinical pregnancy rate, the number of oocytes retrieved, the fertilization and implantation rate and the incidence of OHSS. All parameters were assessed as per patient.
Results. Amongst the six studies that met the inclusion criteria, only two were randomized controlled trials (RCTs). The doses compared were 2000, 3300, 5000, 10,000 and 15,000 IU of u-hCG. Meta-analysis was not conducted due to the lack of sufficient number of RCTs and heterogeneous variables in these sparse studies. The majority of studies concluded that the clinical outcomes are similar between women receiving 5000 compared with 10,000 IU of u-hCG. However, only two studies commented on the number of women who developed OHSS. The incidence of OHSS was not eliminated in the high-risk population even with lower dose of u-hCG.
Conclusions. There is no evidence that by reducing the dose of u-hCG we significantly decrease the risk of OHSS. Until large-scale RCTs that would assess not only both the clinical effectiveness but also the adverse effects related to various doses of u-hCG are conducted, the management of those women and especially of the high-responders needs to be individualized.

P13: Empty follicular syndrome

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Recurrent EFS is a rare phenomenon, its impact very serious and devastating for the patient.

We herein report a case of genuine 'empty follicular syndrome' in a woman in whom no oocytes was retrieved in three consecutive IVF cycles. Our patient is a 28-year-old woman with primary

unexplained subfertility of 5 years duration. She underwent three IVF cycles using the long down-regulation protocol. In all three cycles, apparently good ovarian response was observed. Prior to egg collection, HCG was given at three different times in each cycle (34, 32 and 36 h) and on each occasion, HCG was used from three different batches. In spite of repeated flushing of all follicles by an experienced operator, no oocytes were retrieved. The woman also had an ovarian biopsy and chromosomal analysis, which were normal.

Our case suggests that genuine EFS does exists. The possible underlying cause of EFS in our case seems to be ovarian aging in which the granulosa cells retain some responsiveness but oocyte can no longer develop adequately. The report highlights the fact that EFS cannot be predicted by the pattern of ovarian response to super ovulation with endocrinology or sonographically. Consequently, the diagnosis of EFS remains a retrospective one. There is a great need to find out the cause of this puzzling condition and possibly a way of predicting it.

P14: Optimisation of endometrial receptivity in medicated frozen embryo replacement cycles. The long proliferative phase revisited

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Background. Medicated frozen embryo replacement cycles (FERCs) using oestrogen and progesterone supplementation with or without prior pituitary down-regulation yield good results.

Objective. To examine the influence of the duration of oestrogen supplementation on outcome of medicated FERCs with or without pituitary down-regulation.

Methods. Analysis of 1124 cycles of medicated FERCs in which oestrogen supplementation was started in 701 cycles after pituitary down-regulation using buserelin nasal spray (group A) and in 423 cycles without prior pituitary down-regulation (group B). Cycles in each group were subdivided depending on duration of oestrogen supplementation given to achieve adequate endometrial thickness (<21 days and ≥ 21 days).

Results. In group A, the implantation, clinical pregnancy and ongoing pregnancy/delivery rates remained similar in cycles where oestrogen supplementation exceeded 20 days ($n=206$) compared with cycles where oestrogen supplementation was 20 days or less ($n=495$) [20.1% *vs*. 19.7%, $P=0.87$; 29% *vs*. 28%, $P=0.85$ and 26% *vs*. 23%, $P=0.47$]. However, in group B, there was a significant decline in the implantation, clinical pregnancy and ongoing pregnancy/delivery rates when oestrogen supplementation exceeded 20 days ($n=203$) compared with cycles where oestrogen supplementation was 20 days or less ($n=220$) [10% *vs*. 17%, $P=0.01$; 16% *vs*. 26%, $P=0.03$; 14% *vs*. 23%, $P=0.03$, respectively]. Multivariate logistic regression analysis showed that extending the duration of oestrogen supplementation beyond 20 days was a significant factor affecting outcome in medicated FERCs started without pituitary down-regulation (adjusted OR 0.53, 95% CI 0.32–0.89, $P=0.016$).

Conclusion. Pituitary down-regulation prior to medicated FERCs protects the endometrium from the effects of prolongation of the proliferative phase beyond 20 days.